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1629-Pos Board B539
ATP Hydrolysis Energy Transfer in the Profilin-Mediated Actin Polymerization
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Profilin regulates actin polymerization in cells playing important role in cell motility and division. Actin polymerization involves ATP hydrolysis which occurs both in the absence and the presence of profilin. There is a hypothesis suggested in literature that profilin promotes actin polymerization through direct transfer of the energy of this spontaneous hydrolysis to polymerization utilizing tight coupling of the hydrolysis and corresponding polymerization events. Recently we suggested an alternative hypothesis based on an indirect energy transfer and pointed out that recent experimental and theoretical findings require re-evaluation of the direct transfer hypothesis.
Our thermodynamically rigorous model of actin steady state dynamics in the presence of profilin describes all events in terms of chemical reactions and allows both energy transfer mechanisms, each corresponding to certain ranges of the rate constants (parameters) for these reactions. In fact, the difference between the two mechanisms is defined by a single ratio r of the two rates: the rate of hydrolysis by the profilin-and-ATP-bound subunit at the filament end, and the rate of dissociation of this subunit (in complex with profilin) from the end. The direct transfer cannot exist unless $r>1$ while indirect transfer can occur at any r.
Our model predicts specific shapes for the dependence of actin critical concentration on profilin concentration depending on the ratio r. For values $r>1$, curves have peaks, which persist for wide ranges of other model parameters. Our experimental dependence (obtained with our new technique) shows no peak but a steady decline, contrary to the direct transfer hypothesis. This result provides strong support for our indirect transfer hypothesis.
Moreover, using our theory and routine experimental techniques we determined relative activities of the two specific molecular mechanisms of profilin action (both based on indirect transfer) predicted earlier with our model.

1630-Pos Board B540
The Effect of Toxofilin on the Structure of Monomeric Actin
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The actin cytoskeleton of eukaryotic cells plays a key role in many processes, including motility and cytokinesis. The structure and dynamics of the cytoskeleton are regulated by a large number of proteins that interact with monomeric and/or filamentous actins.
Toxoplasma gondii is an intracellular parasite, which can utilise the actin cytoskeleton of the host cells for their own purposes. One of the expressed proteins of *T. gondii* is the 27 kDa-sized toxofilin. The 245 amino acid long protein is a monomeric actin-binding protein involved in the host invasion. It can bind to actin monomers and to the ends of the actin filaments as well. The protein has three actin-binding sites which makes it capable to interact with an antiparallel actin dimer.
In our work we studied the effect of the actin-binding site of toxofilin$_{69\text{-}196}$ on the monomeric actin. We determined the affinity of toxofilin to the actin monomer with fluorescence anisotropy measurement (K_D = 1.3 μM). The flouresscence of the actin bound ϵ-ATP was quenched with acrylamide in the presence or absence of toxofilin. In the presence of toxofilin the accessibility of the bound ϵ-ATP decreased, which indicates that the nucleotide binding cleft is shifted to a more closed conformational state.
The results of the completed experiments can help us to understand in more details what kind of cytoskeletal changes can be caused in the host cell during the invasion of the host cells by intracellular parasites.

1631-Pos Board B541
***Saccharomyces Cerevisiae* Glycolytic Enzymes are Stabilized by Association with Actin**
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Adela Mújica Miranda, Salvador Uribe Carvajal.
The cell contains constant concentrations of solutes and macromolecules except during stress, when compatible solutes accumulate in the cytosol. Molecular crowding in the cell results in protein association that allows the channeling of intermediates and thus increasing metabolic efficiency. Multienzymatic complexes (or metabolons) are anchored in a dynamic cytoskeleton. It is suggested that the efficiency of cellular metabolism depends on the enzymatic organization. In addition, metabolon probably protect enzymes in a metabolic pathway from the deleterious effects of stress. It was decided to examine whether glycolytic enzymes associate with actin and whether association confers higher stability to the different enzymes. Enzyme association was assessed by co-immunoprecipitation of actin with glycolytic enzymes in the presence or absence of compatible solutes. The whole fermentation pathway was also assayed in the presence of increasing compatible solutes. Actin stabilized the glycolytic pathway making a more efficient pathway even in the presence of a compatible solute. By contrast, depolymerization of actin did not affect fermentation.

1632-Pos Board B542
Acyl Chain Specificity of the Inhibition of Actin Polymerization by the Interaction of Lysophosphatidic Acid and Villin
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Lysophosphatidic acid binds specifically to villin resulting in the inhibition of villin-induced polymerization of actin (Tomar; George; Mathew and Khurana (2009) J. Biol. Chem. 284, 35278). We have identified an amino acid pattern contributing to the arachidonoyl specificity of several proteins (Shulga, Topham, Epand (2010), submitted). The protein villin contains this pattern and was therefore studied for its specificity for arachidonoyl binding. The binding affinity of lysophosphatidic acid to villin was studied using quenching of the intrinsic Trp fluorescence of this protein by lysophosphatidic acid. We observed that 1-arachidonoyl-lysophosphatidic acid bound more tightly to villin than did 1-oleoyl-lysophosphatidic acid. The region of villin expected to confer arachidonoyl specificity comprises residues 4-18. A mutant of villin was constructed in which residues 4-14 were deleted. Using this mutant villin, there is very little difference in binding affinity between the two species of lysophosphatidic acid. This is a result of a loss of affinity for the arachidonoyl form in the mutant and little change in the binding of the oleoyl form. This is consistent with this domain conferring acyl chain specificity to the lipid interactions of villin. We also determined the effects on actin polymerization. Actin polymerization was promoted to similar extents by either the wild type villin or the deletion mutant. However, this is inhibited by lysophospatidic acid with the arachidonoyl form being more potent than the oleoyl and this inhibition is greater with the wild type protein than with the mutant. These results are consistent with the binding studies and demonstrate acyl chain specificity for this regulation of cytoskeletal rearrangements. The preference for the arachidonoyl form suggests a relationship of this mechanism of regulation of actin polymerization to the lipid intermediates of the PI-cycle.

1633-Pos Board B543
***O*-GlcNac Modification of Human Cardiac α-Actinin**
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Steven B. Marston.
We measured *O*-GlcNAcylation of myofibrillar proteins from human hearts by two assays: (1) Western blotting with an antibody (CTD110.6) specific to *O*-GlcNAc modified proteins and (2) specific enzymatic labelling of *O*-GlcNAc with *N*-acetylgalactosamine (UDP-GalNAz) by mutant enzyme Y289L beta-1,4-galactosyltransferase (Y289L GalT), which allows coupling of tetramethylrhodamine (TAMRA) fluorescent tag for direct imaging following SDS-PAGE. In every sample, the predominant modified protein was alpha-actinin, which made up $65 \pm 4.48\%$ of the enzymatically labelled myofibrillar proteins. We found that alpha-actinin exists as two bands on 12% SDS-PAGE. EA-53 antibody to alpha-actinin revealed that the faster migrating alpha-actinin band is on average $<1.4 \pm 0.31\%$ of the slower migrating band. Interestingly, CTD110.6 showed signals from both bands with similar intensities (mean ratio=1.02 ± 0.10), indicating that *O*-GlcNAc modification is more abundant in the minor band (>80x more concentrated). This was confirmed by the enzymatic labelling method; *O*-GlcNAc was often not detectable in the slower migrating band, with an average ratio of faster/slower migrating band of 2.45 ± 0.49. Further tests showed that the higher mobility band was not a degradation product of the lower mobility band. *O*-GlcNAc can be removed from the myofibril proteins by beta-N-acetylglucosaminidase. Using CTD110.6 detection, increasing enzyme concentration decreased the faster migrating band to near zero, but the slower migrating band did not show the same trend, suggesting that the antibody may cross react slightly with unmodified alpha-actinin. We conclude that *O*-GlcNAcylation is highly concentrated in the low abundance faster migrating species of alpha-actinin. The possibility that the two bands represent two isoforms of alpha-actinin differentially modified by the potentially regulatory *O*-GlcNAc modification is being investigated.